J. Clin. Chem. Clin. Biochem.
Vol. 25, 1987, pp. 869–871

Half-Lives of *L*-[^{35}S]Methionine and *L*-[^{3}H]Fucose of Transferrin in the Serum of Rats

By *D. Josić*

Institut für Molekularbiologie und Biochemie der Freien Universität Berlin, Berlin, West Germany

R. Tauber

Institut für Klinische Chemie und Klinische Biochemie, Universitätsklinikum Charlottenburg der Freien Universität Berlin, Berlin, West Germany

W. Hofmann, J. Mauck and *W. Reutter*

Institut für Molekularbiologie und Biochemie der Freien Universität Berlin, Berlin, West Germany

(Received August 31/October 19, 1987)

Summary: The half-lives of ^{35}S-labelled *L*-methionine and of ^{3}H-labelled *L*-fucose of serum transferrin of rats were measured in pulse-chase experiments in vivo. Both *L*-[^{35}S]methionine and *L*-[6-^{3}H[fucose disappeared from transferrin with nearly the same half-lives of 33.8 h and 36.5 h, respectively. The data show that in this major serum glycoprotein peripheral carbohydrates and the protein moiety are degraded as a unit.

Introduction

It is a general feature of glycoproteins of the liver plasma membrane, that their peripheral carbohydrates are degraded several times faster than the polypeptide moieties (1–4). *L*-Fucose is split off from the glycoproteins especially rapidly as demonstrated for all plasma membrane glycoproteins of rat liver studied so far (1–3). The intramolecular heterogeneity of degradation of membrane glycoproteins seems to be involved in membrane and receptor recycling (5). This assumption is supported by the recent finding that the transferrin receptor (6) and receptor-bound transferrin (7) are both reglycosylated.

However, studies on the intramolecular turnover of *L*-fucose have so far been restricted to membrane-integrated glycoproteins of the liver. Therefore, the aim of the present paper was to study the turnover of a glycoprotein synthesized by the liver that is not inserted into the plasma membrane but secreted into the serum. Transferrin was used as a model glycoprotein since it is a major liver-derived serum glycoprotein.

Materials and Methods

Animals

Male Wistar rats (Ivanovas, Kisslegg, F. R. G.) weighing 180–200 g were fed on a commercial diet (Altromin, Altromin GmbH, Lage-Lippe, F. R. G.) containing 18–20% of protein, and water ad libitum.

Chemicals and radioactively labelled compounds

All chemicals were of analytical grade and were purchased from E. Merck AG (Darmstadt, F. R. G.) or Sigma (St. Louis, U. S. A.). Eupergit-protein A (30N) support for immunoaffinity high performance liquid chromatography (IA-HPLC) was purchased from Röhm Pharma GmbH (Weiterstadt, F. R. G.). The corresponding columns were packed by Bischoff Analysentechnik (Leonberg, F. R. G.). *L*-[^{35}S]Methionine (38.4 PBq/mol) and *L*-[6-^{3}H]fucose (0.48 PBq/mol) were obtained from the Radiochemical Centre (Amersham, Bucks., England). X-Ray film AR-5 was from Kodak.

Polyacrylamide gel electrophoresis

Sodium dodecylsulphate polyacrylamide gel electrophoresis was performed according to the procedure of *Laemmli* (8). For fluorography gels were processed according to *Bonner & Laskey* (9) and exposed to Kodak AR-5 X-ray film at −70 °C.

Isolation of rat serum transferrin by immunoaffinity high performance liquid chromatography

Serum was obtained by coagulation of blood at room temperature and subsequent centrifugation at 1000 g for 15 min. Albumin and IgG were removed from the serum by chromatography on a affi-blue-gel column (BioRad, München, F. R. G.) or on a Eupergit-protein A (30N) 4.6 × 60 mm HPLC column, as previously described (10). Subsequently, transferrin was isolated by immunoaffinity high performance liquid chromatography using polyclonal rabbit anti-transferrin antibodies (Cappel, Cochranville, PA. 19330, U. S. A.) immobilized in a protein A-HPLC column as outlined in l. c. (11). Transferrin was eluted from the column with 0.2 mol/l glycine hydrochloride, pH 2.7 (11).

Determination of half-lives

In each experiment twelve rats were labelled in vivo by intraperitoneal injection of either 92.5 MBq of *L*-[6-^{3}H]fucose (specific radioactivity 0.48 PBq/mol) or 74 MBq of *L*-[^{35}S]methionine (specific radioactivity 38.4 PBq/mol) per kg body weight. Two hours after injection of the label, and then every 12 hours, 500 mg of the respective unlabelled compound per kg of body weight were injected as a chase. At 12, 24, 36 and 48 hours rats were sacrificed and 5 to 6 ml of blood were collected. Transferrin was isolated from serum by IA-HPLC as described above and split into three portions. Two equal portions were separated on two parallel SDS polyacrylamide gels: one gel was fixed, stained with Coomassie Blue and processed for fluorography. The second unstained gel was taken for protein determination. The transferrin band was cut out, eluted with bidist. water and protein was determined by the method of *Lowry* et al. (12) with human transferrin as a standard. The third portion of isolated transferrin was used for determination of radioactivity by direct counting in 10 ml Unisolve 1 scintillator (Koch Light, Colnbrook, Bucks., England). Independently, radioactivity of the transferrin bands was quantitated by scanning of the fluorograms.

Half-lives of degradation were calculated from the decrease of specific radioactivities of transferrin as described in l. c. (2).

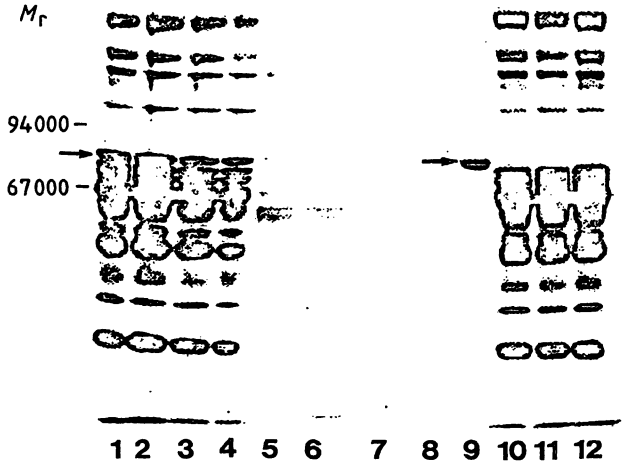

Fig. 1. Isolation of transferrin from [^{35}S]methionine-labelled serum by immunoaffinity HPLC.
Samples (20 µl from each fraction) were analysed by SDS polyacrylamide gel electrophoresis and fluorography.
lines 1 – 4: serum before analysis
lines 5 – 8: non-specifically bound material washed out with 0.1 mol/l Na borate buffer, pH 8.0, containing 1 ml/l Triton X-100.
line 9: specifically bound serum transferrin eluted with 0.2 mol/l glycine hydrochloride, pH 2.7.
line 10 – 12: material not bound to the column.
Transferrin bands are marked with arrows. For details see Material and Methods.

Results

Serum transferrin (cf. fig. 1, line 9) was quantitatively bound to the protein A-anti-transferrin column. The transferrin band which is clearly visible in the serum before chromatography (cf. fig. 1, lines 1 – 4, arrow), appeared not in immunoaffinity chromatography fractions (cf. fig. 1, lines 10 – 12, arrow), except the fraction eluted from the column with 0.2 mol/l glycine hydrochloride, pH 2.7, in which only the transferrin band was radioactively labelled (cf. fig. 1, line 9).

In vivo half-lives of *L*-fucose and the polypeptide moiety of serum transferrin were measured in pulse-chase experiments. The fluorographic analysis of transferrin isolated by immunoaffinity chromatography from the serum at different times after labelling of animals with *L*-[6-^{3}H]fucose showed that the specific radioactivity of transferrin clearly decreased within 48 h (fig. 2). Analysis of transferrin labelled with *L*-[^{35}S]methionine gave the same result. Loss of protein-bound radioactivity fitted first-order kinetics (fig. 3). Calculated half-lives were 36.5 h for *L*-fucose and 33.8 h for *L*-methionine, respectively (fig. 3).

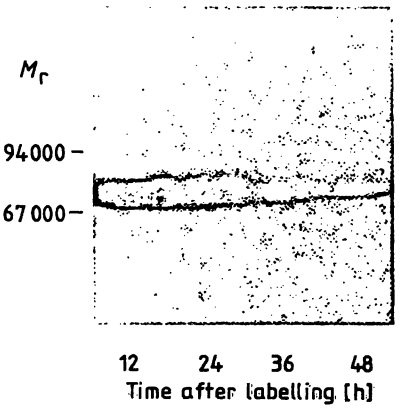

Fig. 2. Disappearance of *L*-[6-^{3}H]fucose in rat serum transferrin.
Transferrin was isolated by immunoaffinity chromatography from serum obtained 12, 24, 36 and 48 h after in vivo labelling of rats with *L*-[6-^{3}H]fucose. Immunoaffinity chromatography fractions were analysed by SDS polyacrylamide gel electrophoresis and fluorography.

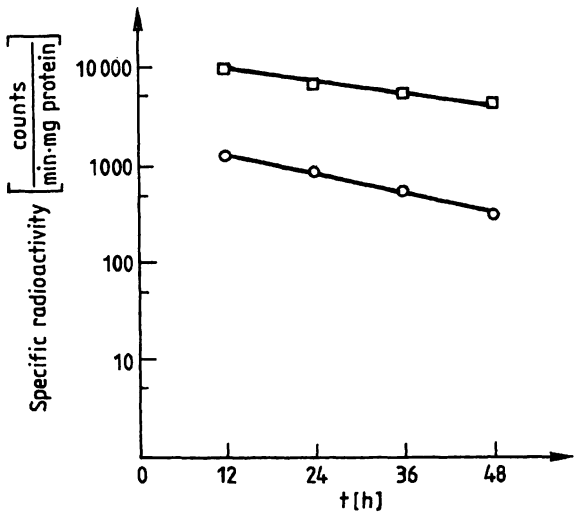

Fig. 3. Half-life of *L*-[6-^{3}H]fucose and *L*-[^{35}S]methionine in rat serum transferrin.
Transferrin was isolated by immunoaffinity chromatography from serum obtained 12, 24, 36 and 48 h after labelling of rats with either *L*-[6-^{3}H]fucose (□) or *L*-[^{35}S]methionine (○) (see Methods). Specific radioactivities calculated from liquid scintillation counting and protein determination were plotted semilogarithmically versus time. Half-lives were calculated from the decrease of specific radioactivity: 36.5 h for *L*-fucose, 33.8 h for *L*-methionine. For details see Material and Methods. Regression lines were determined by the least-squares method. The correlation coefficients are 0.999 for the half-life of protein backbone (○—○) and 0.997 for protein-bound fucose (□—□).

Discussion

In rat serum transferrin in vivo half-lives of *L*-fucose and *L*-methionine were almost identical evidencing that the peripheral carbohydrates and the polypeptide moiety of this serum glycoprotein are degraded as a unit. By contrast, *L*-fucose of plasma membrane glycoproteins is degraded with half-lives three to four times shorter than those of the protein backbone of the glycoproteins (1—4). The different turnover of *L*-fucose in plasma membrane and serum glycoproteins indicates that peripheral carbohydrates have different functions in these two glycoprotein classes. There is increasing evidence that the rapid removal of peripheral carbohydrates of plasma membrane glycoproteins and a subsequent reglycosylation are involved in membrane-specific processes, e. g. in membrane and receptor recycling, or in the intracellular movement of membrane glycoproteins (6, 13, *Kreisel* et al., submitted). By contrast, for serum glycoproteins it is well known from the studies of *Pricer & Ashwell* (14) that their metabolic stability depends on the intact structure of their carbohydrates.

Acknowledgement

This work was supported by the Deutsche Forschungsgemeinschaft (Bonn-Bad Godesberg), the Fonds der Chemischen Industrie (Frankfurt) and the Boehringer Ingelheim Fonds (Stuttgart).

References

1. Kreisel, W., Volk, B., Büchsel, R. & Reutter, W. (1980) Proc. Natl. Acad. Sci. U.S.A. *77*, 1828—1831.
2. Tauber, R., Park, C. S. & Reutter, W. (1983) Proc. Natl. Acad. Sci. U.S.A. *80*, 4026—4029.
3. Baumann, H., Hou, E. & Jahreis, G. P. (1983) J. Cell Biol. *96*, 139—150.
4. Hanski, Ch., Evans, W. H. & Reutter, W. (1984) Eur. J. Cell Biol. *33*, 123—126.
5. Reutter, W. & Tauber, R. (1983) Gann Monogr. Cancer Res. *29*, 59—65.
6. Snider, M. D. & Rogers, O. (1985) J. Cell Biol. *100*, 826—834.
7. Regoeczi, E. P., Chindemi, A., Debanne, M. T. & Charlwood, P. A. (1982) Proc. Natl. Acad. Sci. U.S.A. *79*, 2226—2230.
8. Laemmli, U. K. (1970) Nature (London) *227*, 680—685.
9. Bonner, W. M. & Laskey, R. A. (1974) Eur. J. Biochem. *46*, 83—88.
10. Josić, D., Hofmann, W., Habermann, R., Becker, A. & Reutter, W. (1987) J. Chromatogr. *397*, 39—46.
11. Josić, D., Hartl, R., Hofmann, W. & Reutter, W. (1987) Proc. 11th Internat. Symposium-HPLC 87, book 1, p. 78, Amsterdam.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. *193*, 265—275.
13. Snider, M. D. & Rogers, O. (1986) J. Cell Biol. *103*, 265—275.
14. Pricer, W. E. & Ashwell, G. (1971) J. Biol. Chem. *246*, 4825—4833.
15. Klausner, R. D., van Renswoude, J., Ashwell, G., Kemp, K. & Bridges, K. (1983) J. Biol. Chem. *258*, 7415—7424.

Prof. Dr. W. Reutter
Institut für Molekularbiologie und Biochemie
der Freien Universität Berlin
Arnimallee 22
D-1000 Berlin 33 (Dahlem)